MELATONIN-NITRIC OXIDE CROSSTALK AND THEIR ROLE IN REDOX HOMEOSTASIS IN SOME CYANOBACTERIA GROWING UNDER HEAVY METAL STRESS

Synopsis

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INTRODUCTION

Being the first known oxygenic photosynthetic microbes on Earth, cyanobacteria have produced oxygen in the atmosphere for the last three billion years (Sánchez-Baracaldo & Cardona, 2020). They have converted reducing atmosphere to oxidizing making it suitable for all life forms. They also play a significant role in the biomass of the aquatic environment and may be found in freshwater, seawater, or wetlands. They exist in a variety of forms, from unicellular to filamentous and colonial (Leaf et al., 2020). Being the main producers in aquatic ecosystems they involve three main photosynthetic complexes, photosystem I (PS I), photosystem II (PS II), and phycobilisome (PBS). These complexes acquire light energy between 400 and 700 nm (Bryant et al., 2018). Furthermore, they act as facilitators in the nitrogen fixation process. These days, there is increased focus on cyanobacteria due to their significant economic role. They are employed in the pharmaceutical sectors for the manufacture of several bioactive chemicals, such as vitamins, enzymes, antibiotics, hormones, and others, as well as for the breakdown of waste materials, metal homeostasis, and the suppression of the development of perilous microbes. Cyanobacteria also serve as a source of bioenergy production (Chittora et al., 2020). Cyanobacteria are also employed in modifying the physicochemical characteristics of soil, as well as their characteristics associated with biosorption and bioremediation (Singh, 2020). As natural colorants and dyes for food coloring and other uses, cyanobacterial pigments are commercially valuable (Perera et al., 2023). Several cyanobacterial strains, including *Aphanothece sacrum*, *Nostoc commune*, *Nostoc flagelliforme*, *Nostoc punctiforme*, *Spirulina platensis*, and *Spirulina maxima* (formerly recognized as *Arthrospira sp*.), are consumed since they are an excellent source of proteins, vitamins, and antioxidants (Panjiar et al., 2017). In recent years, there has been a significant global focus on environmental degradation resulting from unconstrained developmental activities like industrialization and urbanization. Aquatic ecosystem pollution is constantly rising as a result of waste and discharges from various sectors that include high levels of hazardous heavy metals (Tiwari et al., 2019). Heavy metal adversely affects the growth of cyanobacteria by inhibiting its growth and hinders maximum utilization of its bioactive compounds in different sectors.

Heavy metal contamination is an increasing concern for both the environment and human health, particularly in emerging nations like India. Anthropogenic activities such as electroplating, mining,

smelting, pesticide and fertilizer discharges, bio-solids, municipal sewage/sludge, and the textile and paint industries have become the primary contributors to metal pollution. Heavy metals are found in hazardous amounts in both terrestrial and aquatic water bodies due to industrial waste discharge. Heavy metals comprise Cadmium (Cd), Lead (Pb), Mercury (Hg), Chromium (Cr), and Arsenic (As) as the main environmental contaminants, especially in places with significant anthropogenic pressure, and are detrimental even at lower concentrations (Mishra et al., 2019; Middepogu, 2016). These pollutants have an adverse effect on the development and survival of the aquatic ecosystem's primary producers (Tiwari et al., 2019). Organisms are greatly harmed by elevated concentrations of these heavy metals, and the degree of toxicity mostly relies on the kind and duration of exposure (Rehman et al., 2018). Growth and development of cyanobacteria is also adversely affected by heavy metals, and the reduction in growth has been linked to a decrease in photosynthetic pigments, nutrient intake, photosynthesis, and nitrogen-fixation efficiency (Pandey et al., 2022). Reactive oxygen species (ROS) production, which disassembles macromolecules including protein, DNA, and lipids, is a prevalent endpoint used to evaluate the degree of toxicity. They inactivate several enzymes that are involved in various physiological and biochemical processes and also damages the structure of cellular membranes (Singh et al., 2016). Recent research suggests that exogenous application of growth-promoting hormones such as kinetin (Tiwari et al., 2020), indole acetic acid (IAA), Ca^{2+} as a secondary messenger (Ahad and Syiem, 2019), melatonin (Yuan et al., 2022) and NO a signaling molecule (Tiwari et al., 2019) has significantly contributed in enhancing the tolerance of cyanobacteria to different abiotic stresses.

Melatonin (MEL) or N-acetyl-5-methoxytryptamine, is a novel hormone, a widely distributed molecule found in microbes, plants, and mammals (Xie et al., 2022). Melatonin has been recognized as a unique type of metabolic regulator in the biological kingdoms within the past several years. It can increase a plant's resistance to a variety of abiotic conditions, including extreme heat (Jahan et al., 2019), low temperatures (Li et al., 2017), salt (Zhan et al., 2019), and heavy metals (Altaf et al., 2023). Melatonin is a potent scavenger of reactive oxygen species (ROS) in crops, vegetables, and algae, including H_2O_2 and $O_2^{\bullet-}$ (Zhang et al., 2018). It has been shown that exogenous melatonin increases tolerance to adverse environmental conditions by increasing the activities of antioxidant enzymes,

detoxifying reactive oxygen species (ROS) to acceptable levels, and controlling the transcription levels of genes linked to the antioxidant system in organisms (Zhang et al., 2022). The majority of research has proven how melatonin helps plants recover from various abiotic stresses. Less is known about its possible impacts on cyanobacteria. However, it is produced in cyanobacteria but its signaling and mode of action is unclear as to how it affects the growth of cyanobacteria.

Likewise, exogenous administration of signaling molecules (e.g., hydrogen peroxide (H_2O_2), nitric oxide (NO) and hydrogen sulphide (H_2S)) is a highly effective method for improving plant tolerance to environmental challenges (Antoniou et al., 2016). Among these, Nitric oxide (NO) is a widely distributed, lipophilic physiologically active signaling molecule that is currently believed to have the potential to function as an intermediate in controlling how plants react to stress (Asgher et al., 2017; Begara-Morales et al., 2018). Numerous studies have documented the endogenous production of NO in lichens, algae, higher plants, and certain non-flowering plants including gymnosperms and ferns (Tiwari et al., 2019). Thanks to its wide range of physiological roles and omnipresence, NO has gathered increased attention. Additionally, NO is well-known for its active roles in plant growth, gene expression, defense mechanisms (such as the production of antioxidant enzymes), and stomatal movement in response to stress (Verma et al., 2020). Studies on the impact of NO in cyanobacteria have shown to enhance its growth (biomass), photosynthetic activity and pigments. ROS significantly decreased under the impact of NO and antioxidant defense system enhanced extremely (Verma & Prasad, 2021).

Prior research showed that melatonin stimulates plants to produce and accumulate NO, which functions as an antioxidant and governs other defensive mechanisms within plants (Okant & Kaya, 2019). Under abiotic stress, MEL and NO can both interact at different levels, beginning with their biosynthetic pathways and triggering specific signaling responses in plants (Pardo-Hernández et al., 2020). Recent research has shown that the combination of MEL with NO produces NOMela, or N-nitrosomelatonin, which is expected to have roles in plant morphophysiological activity (Martínez-Lorente et al., 2022). MEL is capable of interacting with NO, which regulates different physiological and biochemical processes in plants (Zhu et al., 2019) and algae (Ding et al., 2018) ultimately leading to biomass accumulation and productivity. So far, no research has been conducted on cyanobacteria,

so we plan to investigate integrated crosstalk between melatonin and nitric oxide.

Hypothesis:

We hypothesize that this research will uncover insightful mechanisms by which melatonin regulates heavy metal stress in cyanobacteria. Additionally, we aim to elucidate the potential crosstalk between melatonin and nitric oxide in this regulatory process. Our research will provide a comprehensive understanding of how melatonin mitigates the detrimental effects of heavy metals, exploring its interactions at the physiological and biochemical levels. By investigating the interplay between melatonin and nitric oxide, we hope to reveal insights into their synergistic roles and potential pathways involved in stress response and cellular protection.

Objectives:

To understand the integrated crosstalk between melatonin and NO under heavy metal stress in some cyanobacteria using **Sodium nitroprusside** (SNP), donor of NO, **N(ω)-nitro-L-arginine methyl ester** ($_{L}$NAME), inhibitor of NO and **2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide** (cPTIO) scavenger of NO, following objectives have been set forth:

1. Analysis of growth pattern (biomass accumulation and culture absorbance), photosynthetic pigments, and PSII photochemistry in selected cyanobacteria grown in the above-mentioned conditions.
2. Analysis of inorganic nitrogen content and enzymes involved in nitrate and ammonium assimilation in selected cyanobacteria.
3. Evaluation of oxidative damage and anti-oxidant metabolites in selected cyanobacteria.
4. Quantification of biomolecules and bioactive compounds regulated by melatonin under heavy metal stress in selected cyanobacteria.

Significance of this study will be as follows:

- The present study will be able to address agricultural challenges as well as industrial challenges by exploring the interactive role of melatonin and nitric oxide in cyanobacteria under heavy metal stress.
- Melatonin's mechanism of action in improving biomass accumulation and production of bioactive compounds in cyanobacteria under heavy metal-stressed environments can lead to increased

worldwide productivity and applications in various industries.

- The interactive role of melatonin and nitric oxide in enhancing lipid productivity, which could efficiently be utilized to generate biofuels (generation III) for a sustainable environment.
- This early-stage research will help validate future research in this area.

REVIEW LITERATURE

Impact of heavy metals on growth and photosynthetic machinary: A number of authors have noticed a decline in cyanobacteria biomass accumulation when exposed to heavy metals (Patel et al., 2018). In addition to growth, photochemistry is facilitated by the photosynthetic pigment contents, which include phycobiliproteins (PBPs), carotenoids (Car), and chlorophylls (Chl). These pigments absorb light energy from the sun and transport it to the reaction centres (Pandey et al., 2022). Tiwari et al. (2018) and Patel et al. (2019) reported that whenever *Nostoc* is exposed to Cr/As, its chlorophyll concentration decreases, which is linked to reduction in photosynthetic activity. In addition to lowering pigment concentrations, heavy metals also reduce thylakoids' surface area, which inhibits photosynthetic activity by altering QB's role as PS II's major electron acceptor. Thus, reduces PS II activity (Middepogu, 2016).

Impact of heavy metals on Nitrogen metabolism: Many proteins, nucleic acids, and enzymes that control cyanobacterial development require nitrogen as a necessary component. Excessive environmental concentrations of heavy metals drastically lower nitrogen intake and change the functions of N assimilatory enzymes (Dubey et al., 2021). Awasthi and Rai, 2005 reported that heavy metals such as Ni, Cd, and Zn caused a decrease in nitrate absorption in *Scendesmus quadricauda*. Under Cr stress, decreased inorganic nitrogen absorption causes GS/GOGAT to decline (Tiwari et al., 2021). Similarly, Rai et al., 1998 reported inhibition of nitrate uptake in *Anabaena* when exposed to heavy metal stress.

Impact of heavy metals on oxidative stress biomarkers and antioxidant systems: Patel et al. 2021 reported significant increase in the level of SOR and H_2O_2 in *Nostoc muscorum* and *Anabaena* PCC7120 when exposed to As stress. Malondialdehyde (MDA) was used to measure the ROS-mediated lipid peroxidation. MDA content was also found to be raised under heavy metal stress in both the species. Enzymatic antioxidants (SOD, POD, CAT and GST) levels were also reported to

increase under heavy metal exposure (Patel et al., 2021).

Impact of heavy metals on status of bioactive compounds and biomolecules: Tiwari et al., 2020 reported that biomolecules (proteins and carbohydrates) content decreased under heavy metal stress. Bioactive compound (exopolysaccharides) was also reported to increase under heavy metal stress.

Impact of melatonin and nitric oxide on growth, physiological and biochemical parameters of cyanobacteria

Yuan et al., 2022 reported that melatonin increased the growth (dry weight) of *Nostoc flagelliforme* when exposed to salt stress. He also reported that melatonin application regulates the photosynthetic pigments and enhance the *Fv/Fm* ratio. Generation of reactive oxygen species under salt stress were also reported to decline by exogenous supplementation of melatonin. Likewise, antioxidant defense system (SOD, POD, CAT, APX, GR) were found to enhance with supplementation of melatonin.

Exogenous supplementation of melatonin significantly enhanced the quantum yield of PS II photochemistry and antioxidant defense system in *Chara australis* and *Chlamydomonas reinhardtii* (Lazar et al., 2013; Zhang et al., 2018). Supplementation of SNP (NO) enhanced the growth of *Nostoc muscorum* ATCC27893 and *Anabaena* PCC7120 as compared to stressed cultures. They also reported that exopolysaccharide and protein contents were also increased. Negative impacts of ROS (H_2O_2, SOR, MDA) were found to decrease with NO application and the antioxidant defense system was enhanced extremely (Verma & Prasad, 2023).

MATERIALS AND METHODS

For the present study, the above said objectives require more sophisticated methods and experimental approaches to prove the relevance of finding and its importance in present day environment. Following standard methodology will be undertaken to fulfill the objectives:

Procurement, growth and maintenance of cyanobacterial strains:

Cyanobacterial cultures will be procured from Centre for Conservation and Utilization of Blue Green Algae (CCUBGA) Division of Microbiology, ICAR-Indian Agricultural Research Institute (PUSA), New Delhi-110012, INDIA, and will be maintained in respective media by employing standard protocols and techniques in laboratory.

Treatment design: Treatment will be conducted when cells will be in the early log phase. The

cultures will be centrifuged and cleansed three times with sterile double distilled water and re-suspended in the respective medium. To know the susceptibility of cyanobacterial strains to selected heavy metal the cultures will be exposed with different concentration of selected heavy metal (HM). Based upon the result of screening, dose will be selected for further study. Further the screening experiment will also be conducted for melatonin (MEL), nitric oxide donor: sodium nitroprusside (SNP), nitric oxide inhibitor: N (ω)-nitro-L-arginine methyl ester ($_L$NAME) and nitric oxide scavenger: 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO).

So, taking into account with the aforementioned doses, experiments will be done with various combinations, like control (untreated), Heavy metal (HM), Melatonin (MEL), Nitric oxide (SNP), HM + MEL, HM+ SNP, SNP+ cPTIO+ HM, SNP+ $_L$NAME+ HM, MEL+ cPTIO+ HM, MEL+ $_L$NAME+ HM and various experimental parameters will be examined.

Growth measurement: Growth or biomass accumulation will be measured in terms of culture absorbance and dry weight (DW). For culture growth (OD) absorbance of treated and untreated cyanobacterial cell will be recorded spectrophotometrically at 750 nm after treatment. For dry weight (DW) the treated and untreated samples will be centrifuged at 4000 g for 10 min and will be washed thrice with double distilled water. Finally, the cells will be oven-dried at 65–75ºC for 48 h and will be weighed using single pan digital balance.

Estimation of Photosynthetic Pigment Contents: Chlorophyll *a*, Carotenoids, and Phycobiliproteins (PBPs) Contents: Quantitative estimation of chlorophyll *a* and carotenoids will be done as per the methods of Porra et al. (1989) and Morton & Goodwin (1956), respectively by using UV-Visible spectrophotometer. Estimation of phycobiliproteins (PBPs) will be done by following the method of Bennett and Bogorad (1973).

PS II photochemistry (JIP-Test): PS II activity of cyanobacteria will be detected by chlorophyll a fluorescence kinetics measurement, which will be examined in the dark adapted cultures treated with heavy metal, SNP (NO donor), MEL, cPTIO (scavenger of NO) and $_L$NAME (inhibitor of NO) using hand held fluorometer (AquaPen AP 100, Photon System Instruments, Czech Republic). The bioenergetics parameters and the energy fluxes parameters will be analyzed according to Strasser et al. (2000).

Intracellular accumulation of heavy metal: The cellular accumulation of heavy metal will be estimated by atomic absorption spectrophotometer (iCE 3000 series, Model 3500 AAS, Thermo scientific, UK).

Measurement of Nitrate (NO_3^-) and Nitrite (NO_2^-) Uptake: The NO_3^- and NO_2^- uptake rate in control and treated cyanobacterial cells will be estimated by calculating the depletion of NO_3^- and NO_2^- from the medium by adopting the methods of Cawse (1967) and Snell and Snell (1949), respectively.

Estimation of Nitrate Assimilating Enzymes: The nitrate reductase (NR) and nitrite reductase (NiR) activity in treated and untreated cultures will be measured by following the method of Herrero et al. (1981 and 1984) and Herrero and Guerrero (1986) respectively.

Glutamine Synthetase (GS) Activity: The GS activity in treated and untreated cultures will be estimated by following the method of Mérida et al. (1991).

Glutamate Synthase Activity: Method of Meers et al. (1970) will be followed for the measurement of NADH-GOGAT.

Glutamate Dehydrogenase Activity: The GDH activity will be quantified according to the method of Chávez and Candau (1991).

Biochemical analysis of oxidative stress biomarkers: Oxidative stress biomarkers: superoxide radicals (SOR; $O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) will be estimated by following the method of Elstner and Heupel (1976) and Velikova et al. (2000), respectively. Moreover, indices of oxidative stress as lipid peroxidation (measured in terms of malondialdehyde; MDA equivalent contents) will be estimated by the method of Heath and Packer (1968).

In-vivo visualization of oxidative stress biomarkers: Further, the cumulative production of $O_2^{\bullet -}$ and H_2O_2 will be quantified in-vivo by staining of cyanobacterial cells with nitro blue tetrazolium (NBT) and 3, 3′ diaminobenzidine (DAB)–HCl, respectively by the method given by Förster et al. (2005). The visualization of MDA equivalents contents will be done by the method given by Pompella et al. (1987) using Schiff's reagent.

Determination of enzymatic antioxidants activity: Superoxide dismutase (SOD) activity will be assayed by following the method of Giannopolitis and Reis (1977) following the photoreduction of

NBT (formation of purple formazone). Peroxidase (POD) activity will be determined as described by Gahagan et al. (1968) and catalase activity by the method of Aebi (1984). Similarly, glutathione-S-transferase (GST) activity will be measured by following the method of Habig et al. (1974).

Ascorbate-glutathione cycle: The method of Nakano and Asada (1981) will be followed for estimation of APX activity in treated and untreated cyanobacterial suspension. The glutathione reductase activity will be assayed by following the method of Schaedle and Bassham (1977). The DHAR activity will be measured by following the method of Nakano and Asada (1981).

Estimation of non-protein thiol (NP-SH) and total phenolic (TPCs): For NP-SH estimation, cultures will be centrifuged and the pellets will be homogenized in sulphosalicylic acid and content will be estimated by taking absorbance at 412 nm by following the method of Ellmann (1959). Total phenolic content (TPC) will be estimated following the method of Waterhouse (2002).

Estimation of proline (Pro) and cysteine (Cys) content: Estimation of proline and cysteine content will be estimated by the method of Bates et al. (1973) and Gaitonde (1967), respectively.

Estimation of total ascorbate (AsA+DHA), reduced ascorbate (AsA), and oxidized ascorbate (dehydroascorbate: DHA): By following the method of Gossett et al. (1994), total ascorbate (AsA+DHA), reduced ascorbate (AsA), and dehydroascorbate (DHA) contents will be determined.

Estimation of reduced glutathione (GSH) and oxidized glutathione (GSSG) and total glutathione (GSH+GSSG): Total, reduced, and oxidized glutathione will be determined by adopting the method of Brehe and Burch (1976) with some modifications.

Estimation of Protein: The cultures will be centrifuged and homogenized with phosphate buffer (pH 7.8), and protein content will be quantified as per the method of Bradford (1976) estimation of protein content will be done by taking absorbance at 595 nm.

Estimation of carbohydrate content: The dried samples (10 mg) will be extracted in HCl, centrifuged at 10,000 g for 10 min, and carbohydrate content will be estimated by following the method of Dubois et al. (1956) and the absorbance will be recorded at 490 nm.

Estimation of Exopolysaccharide Content: The 100 ml treated and untreated cultures will be used for exopolysaccharide estimation by the method of Sharma et al. (2008).

Estimation of Flavonoids: The flavonoid content of samples will be determined according to the

method described by Barku (2013).

EXPECTED OUTCOMES:

The expected outcome of the present study will be helpful in understanding the potential crosstalk between melatonin and nitric oxide in regulating the redox homeostasis in cyanobacteria under heavy metal stress. The integrated crosstalk of melatonin and nitric oxide may enhance the overall growth by improving biomass accumulation and enhancing photosynthetic machinery under heavy metal-stressed environments. This can lead to increased worldwide productivity and applications in various industries. The interactive role of melatonin and nitric oxide will enhance bioactive compounds, lipids and exopolysaccharides production which could efficiently be utilized to generate biofuels (generation III) for a sustainable environment.

REFERENCES:

Aebi, H. (1984). [13] Catalase in vitro. In Methods in enzymology (Vol. 105, pp. 121-126). Academic press.

Ahad, R. I. A., & Syiem, M. B. (2019). Influence of calcium on cadmium uptake and toxicity to the cyanobacterium *Nostoc muscorum* Meg 1. Biotechnology Research and Innovation, 3(2), 231-241.

Altaf, M. A., Hao, Y., Shu, H., Mumtaz, M. A., Cheng, S., Alyemeni, M. N., & Wang, Z. (2023). Melatonin enhanced the heavy metal-stress tolerance of pepper by mitigating the oxidative damage and reducing the heavy metal accumulation. Journal of Hazardous Materials, 454, 131468.

Antoniou, C., Savvides, A., Christou, A., & Fotopoulos, V. (2016). Unravelling chemical priming machinery in plants: the role of reactive oxygen–nitrogen–sulfur species in abiotic stress tolerance enhancement. Current opinion in plant biology, 33, 101-107.

Asgher, M., Per, T. S., Masood, A., Fatma, M., Freschi, L., Corpas, F. J., & Khan, N. A. (2017). Nitric oxide signaling and its crosstalk with other plant growth regulators in plant responses to abiotic stress. Environmental Science and Pollution Research, 24, 2273-2285.

Awasthi, M., & Rai, L. C. (2005). Toxicity of nickel, zinc, and cadmium to nitrate uptake in free and immobilized cells of *Scenedesmus quadricauda*. Ecotoxicology and environmental safety, 61(2),

268-272.

Barku, V. Y. A., Opoku-Boahen, Y., Owusu-Ansah, E., & Mensah, E. F. (2013). Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of Amaranthus spinosus.

Bates, L. S., Waldren, R. P. A., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. Plant and soil, 39, 205-207.

Bennett, A., & Bogorad, L. (1973). Complementary chromatic adaptation in a filamentous blue-green alga. The Journal of cell biology, 58(2), 419-435.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2), 248-254.

Brehe, J. E., & Burch, H. B. (1976). Enzymatic assay for glutathione. Analytical biochemistry, 74(1), 189-197.

Bryant, D. A., & Canniffe, D. P. (2018). How nature designs light-harvesting antenna systems: design principles and functional realization in chlorophototrophic prokaryotes. Journal of Physics B: Atomic, Molecular and Optical Physics, 51(3), 033001.

Cawse, P. (1967). The determination of nitrate in soil solutions by ultraviolet spectrophotometry. Analyst, 92(1094), 311-315.

Chakraborty, N., & Acharya, K. (2017). “NO way”! Says the plant to abiotic stress. Plant gene, 11, 99-105.

Chávez, S., & Candau, P. (1991). An NAD-specific glutamate dehydrogenase from cyanobacteria identification and properties. FEBS letters, 285(1), 35-38.

Chittora, D., Meena, M., Barupal, T., Swapnil, P., & Sharma, K. (2020). Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochemistry and biophysics reports, 22, 100737.

Ding, W., Zhao, P., Peng, J., Zhao, Y., Xu, J. W., Li, T., & Yu, X. (2018). Melatonin enhances astaxanthin accumulation in the green microalga *Haematococcus pluvialis* by mechanisms possibly related to abiotic stress tolerance. Algal research, 33, 256-265.

Dubey, R. S., Srivastava, R. K., & Pessarakli, M. (2021). Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions. In Handbook of plant and crop

physiology (pp. 579-616). CRC Press.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry, 28(3), 350-356.

Elstner, E. F., & Heupel, A. (1976). Inhibition of nitrite formation from hydroxylammoniumchloride: a simple assay for superoxide dismutase. Analytical biochemistry, 70(2), 616-620.

Förster, B., Osmond, C. B., & Pogson, B. J. (2005). Improved survival of very high light and oxidative stress is conferred by spontaneous gain-of-function mutations in Chlamydomonas. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1709(1), 45-57.

Gahagan, H. E., Holm, R. E., & Abeles, F. B. (1968). Effect of ethylene on peroxidase activity. Physiologia plantarum, 21(6), 1270-1279.

Gaitonde, M. K. (1967). A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochemical Journal, 104(2), 627.

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. Plant physiology, 59(2), 309-314.

Gl, E. (1959). Tissue sulfhydryl groups. Arch Biochem Biophys, 82, 70-77.

Gossett, D. R., Millhollon, E. P., & Lucas, M. C. (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop science, 34(3), 706-714.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. Journal of biological Chemistry, 249(22), 7130-7139.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics, 125(1), 189-198.

Herrero Moreno, A., Flores García, E., & García Guerrero, M. (1981). Regulation of nitrate reductase levels in the cyanobacteria *Anacystis nidulans*, *Anabaena sp*. strain 7119, and *Nostoc sp*. strain 6719. Journal of Bacteriology, 145 (1), 175-180.

Herrero, A., & Guerrero, M. G. (1986). Regulation of nitrite reductase in the cyanobacterium *Anacystis nidulans*. Microbiology, 132(9), 2463-2468.

Herrero, A., Flores, E., & Guerrero, M. G. (1984). Regulation of the nitrate reductase level in

Anacystis nidulans: activity decay under nitrogen stress. Archives of biochemistry and biophysics, 234(2), 454-459.

Jahan, M. S., Shu, S., Wang, Y., Chen, Z., He, M., Tao, M., & Guo, S. (2019). Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. BMC Plant Biology, 19, 1-16.

Kanwar, M. K., Yu, J., & Zhou, J. (2018). Phytomelatonin: recent advances and future prospects. Journal of Pineal Research, 65(4), e12526.

Ke, Q., Ye, J., Wang, B., Ren, J., Yin, L., Deng, X., & Wang, S. (2018). Melatonin mitigates salt stress in wheat seedlings by modulating polyamine metabolism. Frontiers in plant science, 9, 914.

Lazar, D., Murch, S. J., Beilby, M. J., & Al Khazaaly, S. (2013). Exogenous melatonin affects photosynthesis in characeae Chara australis. Plant Signaling & Behavior, 8(3), e23279.

Leaf, M. C., Gay, J. S., Newbould, M. J., Hewitt, O. R., & Rogers, S. L. (2020). Calcareous algae and cyanobacteria. Geology Today, 36(2), 75-80.

Li, H., Chang, J., Zheng, J., Dong, Y., Liu, Q., Yang, X., & Zhang, X. (2017). Local melatonin application induces cold tolerance in distant organs of *Citrullus lanatus* L. via long distance transport. Scientific Reports, 7(1), 40858.

Martínez-Lorente, S. E., Pardo-Hernández, M., Martí-Guillén, J. M., López-Delacalle, M., & Rivero, R. M. (2022). Interaction between melatonin and no: Action mechanisms, main targets, and putative roles of the emerging molecule nomela. International Journal of Molecular Sciences, 23(12), 6646.

Meers, J. L., Tempest, D. W., & Brown, C. M. (1970). 'Glutamine (amide): 2-oxoglutarate amino transferase oxido-reductase (NADP)', an enzyme involved in the synthesis of glutamate by some bacteria. Microbiology, 64(2), 187-194.

Mérida Berlanga, Á., Candau Chacón, P., & Florencio Bellido, F. J. (1991). Regulation of glutamine synthetase activity in the unicellular cyanobacterium *Synechocystis sp*. strain PCC 6803 by the nitrogen source: Effect of ammonium. Journal of Bacteriology, 173 (13), 4095-4100.

Middepogu, A. R. (2016). Impact of heavy metal poisoning on cyanobacterial photosynthesis and its detoxification. Innoriginal: International Journal of Sciences, 18-23.

Mishra, S., Bharagava, R. N., More, N., Yadav, A., Zainith, S., Mani, S., & Chowdhary, P. (2019). Heavy metal contamination: an alarming threat to environment and human health. Environmental biotechnology: For sustainable future, 103-125.

Morton, R. A., & Goodwin, T. W. (1956). Carotenoids and vitamin A. British Medical Bulletin, 12(1), 37-43.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and cell physiology, 22(5), 867-880.

Okant, M., & Kaya, C. (2019). The role of endogenous nitric oxide in melatonin-improved tolerance to lead toxicity in maize plants. Environmental Science and Pollution Research, 26, 11864-11874.

Pandey, A., Singh, G., Pandey, N., Patel, A., Tiwari, S., & Prasad, S. M. (2022). Impacts of environmental stress on physiology and biochemistry of cyanobacteria. In Ecophysiology and Biochemistry of Cyanobacteria (pp. 65-89). Singapore: Springer Nature Singapore.

Panjiar, N., Mishra, S., Yadav, A. N., & Verma, P. (2017). Functional foods from cyanobacteria: an emerging source for functional food products of pharmaceutical importance. Microbial functional foods and nutraceuticals, 21-37.

Pardo-Hernández, M., López-Delacalle, M., & Rivero, R. M. (2020). ROS and NO regulation by melatonin under abiotic stress in plants. Antioxidants, 9(11), 1078.

Patel, A., Parihar, P., Tiwari, S., & Prasad, S. M. (2019). Auxin (IAA) up regulates growth, photosynthetic pigment and antioxidant machinery in paddy field cyanobacteria N. muscorum. Think India Journal, 22(17), 3808-3822.

Patel, A., Tiwari, S., & Prasad, S. M. (2018). Toxicity assessment of arsenate and arsenite on growth, chlorophyll a fluorescence and antioxidant machinery in *Nostoc muscorum*. Ecotoxicology and Environmental Safety, 157, 369-379.

Patel, A., Tiwari, S., & Prasad, S. M. (2021). Arsenate and arsenite-induced inhibition and recovery in two diazotrophic cyanobacteria *Nostoc muscorum* and *Anabaena* sp.: study on time-dependent toxicity regulation. Environmental Science and Pollution Research, 28(37), 51088-51104.

Perera, R. M. T. D., Herath, K. H. I. N. M., Sanjeewa, K. A., & Jayawardena, T. U. (2023). Recent reports on bioactive compounds from marine Cyanobacteria in relation to human health

applications. Life, 13(6), 1411.

Pompella, A., Maellaro, E., Casini, A. F., Ferrali, M., Ciccoli, L., & Comporti, M. (1987). Measurement of lipid peroxidation in vivo: a comparison of different procedures. *Lipids*, *22*(3), 206-211.

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 975(3), 384-394.

Rai, L. C., Tyagi, B., Rai, P. K., & Mallick, N. (1998). Interactive effects of UV-B and heavy metals (Cu and Pb) on nitrogen and phosphorus metabolism of a N2-fixing cyanobacterium *Anabaena doliolum*. Environmental and Experimental Botany, 39(3), 221-231.

Sánchez-Baracaldo, P., & Cardona, T. (2020). On the origin of oxygenic photosynthesis and Cyanobacteria. New Phytologist, 225(4), 1440-1446.

Schaedle, M., & Bassham, J. A. (1977). Chloroplast glutathione reductase. Plant physiology, 59(5), 1011-1012.

Sharma, M., Kaushik, A., Bala, K., & Kamra, A. (2008). Sequestration of chromium by exopolysaccharides of *Nostoc* and *Gloeocapsa* from dilute aqueous solutions. Journal of hazardous materials, 157(2-3), 315-318.

Singh, R., Parihar, P., & Prasad, S. M. (2018). Sulfur and calcium simultaneously regulate photosynthetic performance and nitrogen metabolism status in As-challenged Brassica juncea L. seedlings. Frontiers in Plant Science, 9, 374618.

Singh, S. (2020). Biosorption of heavy metals by cyanobacteria: potential of live and dead cells in bioremediation. Microbial bioremediation & biodegradation, 409-423.

Snell, F. D., & Snell, C. T. (1949). Colorimetric Methods of Analyses.

Strasser, R. J., Srivastava, A., & Tsimilli-Michael, M. (2000). The fluorescence transient as a tool to characterize and screen photosynthetic samples. Probing photosynthesis: mechanisms, regulation and adaptation, 25, 445-483.

Tiwari, S., Parihar, P., Patel, A., Singh, R., & Prasad, S. M. (2019). Metals in cyanobacteria:

Physiological and molecular regulation. In Cyanobacteria (pp. 261-276). Academic Press.

Tiwari, S., Patel, A., & Prasad, S. M. (2018). Kinetin alleviates chromium toxicity on growth and PS II photochemistry in *Nostoc muscorum* by regulating antioxidant system. Ecotoxicology and Environmental Safety, 161, 296-304.

Tiwari, S., Patel, A., & Prasad, S. M. (2020). Phytohormone up-regulates the biochemical constituent, exopolysaccharide and nitrogen metabolism in paddy-field cyanobacteria exposed to chromium stress. BMC microbiology, 20, 1-15.

Tiwari, S., Verma, N., Singh, V. P., & Prasad, S. M. (2019). Nitric oxide ameliorates aluminium toxicity in *Anabaena* PCC 7120: Regulation of aluminium accumulation, exopolysaccharides secretion, photosynthesis and oxidative stress markers. Environmental and experimental botany, 161, 218-227.

Velikova, V., Yordanov, I., & Edreva, A. J. P. S. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant science, 151(1), 59-66.

Verma, N., & Prasad, S. M. (2023). Modulation of AsA-GSH cycle by exogenous nitric oxide and hydrogen peroxide to minimize the Cd-induced damages in photosynthetic cyanobacteria. Plant Stress, 10, 100269.

Verma, N., Tiwari, S., Singh, V. P., & Prasad, S. M. (2020). Nitric oxide in plants: an ancient molecule with new tasks. Plant growth regulation, 90, 1-13.

Waterhouse, A. L. (2002). Determination of total phenolics. Current protocols in food analytical chemistry, 6(1), I1-1.

Xie, X., Ding, D., Bai, D., Zhu, Y., Sun, W., Sun, Y., & Zhang, D. (2022). Melatonin biosynthesis pathways in nature and its production in engineered microorganisms. Synthetic and systems biotechnology, 7(1), 544-553.

Yuan, X., An, J., Zheng, T., & Liu, W. (2022). Exogenous melatonin improves salt tolerance mainly by regulating the antioxidant system in cyanobacterium *Nostoc flagelliforme*. PeerJ, 10, e14479.

Zhan, H., Nie, X., Zhang, T., Li, S., Wang, X., Du, X., & Song, W. (2019). Melatonin: a small molecule but important for salt stress tolerance in plants. International Journal of Molecular

Sciences, 20(3), 709.

Zhang, Y., Gao, W., Lv, Y., Bai, Q., & Wang, Y. (2018). Exogenous melatonin confers salt stress tolerance to *Chlamydomonas reinhardtii* (Volvocales, Chlorophyceae) by improving redox homeostasis. Phycologia, 57(6), 680-691.